Interim Progress Report

Richness, Extent, Condition, Reproductive Status and Parasitism of fouling communities on commercial vessels

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Submitted by

Ian Davidson, Gail Ashton & Greg Ruiz

Aquatic Bioinvasion Research & Policy Institute

Portland State University

&

Smithsonian Environmental Research Center

Preamble

Ship biofouling is a significant vector of a taxonomically diverse range of marine organisms. Research has shown that biofouling has been a dominant vector for established nonindigenous species on the West Coast of North America and plays an important role in the invasion history of California (Ruiz et al., submitted). Recent studies have also shown that biofouling continues to transfer a wide-range of organisms in substantial numbers, which demonstrates its continued potency as a modern vector of NIS to bays and estuaries (e.g. Inglis et al., 2010). Evidence suggests that introductions via biofouling continue despite maintenance practices among commercial ships that include dry docking and in-water cleaning at regular intervals.

The biofouling vector literature is dominated by measures of propagule identity and quantity, which are both important components of introduction risk. Analysis of propagule quality has been largely absent, however, even though it is another important metric of introduction outcomes. The reproductive capacity of organisms is particularly relevant for biofouling vectors, because most species must release propagules (in a new generation) because they are not directly released into the recipient environment (like ballast water organisms).

The purpose of this study is to evaluate organism condition more closely while also providing measures of propagule richness and abundance on commercial vessels arriving to the US West Coast. Specifically, we are conducting ship sampling to evaluate the richness and relative abundance of organisms on commercial vessels, with an added focus on the condition of organisms, their reproductive status and their parasite/pathogen loads.

Progress to Date

We have sampled 14 commercial vessels since this project was initiated. Vessel types included eleven cruise ships, two barges, and one tug. Sampling has been conducted using SCUBA divers who take photographs and collect samples that are representative of the biofouling richness in each hull location. Ships were chosen based on their availability and access provided by vessel operators for this project. Prior to each dive, a safety protocol and sampling plan was agreed with each vessel. All dives were conducted from a dive boat and sampling was done on one side of the vessel only (the side not tied to dock).

For cruise ships, sampling incorporated bow thrusters, hull areas around the bow, intakes/holes along the side, stabilizers, stern thrusters, propellers, rudders (or azipods), and hull areas at the stern. Sampling of barges and tugs included bow-to-stern transects as well as evaluations of niche areas including ladder holes for barges and heterogeneous hull structures of tugs. All samples collected during the dive were returned to a lab soon after sampling for initial sorting into different morpho-taxa and condition analysis (e.g. live, dead with tissue, dead).

Only one cruise ship had no detectable invertebrate organisms (live or dead) of the eleven sampled so far. Biofouling invertebrates were recorded on all other cruise ships, with preliminary estimates suggesting tens of thousands of organisms occurred on some ships. The highest richness of morpho-taxa was recorded at bow thruster, mid-ship (including intake/outflow holes), stabilizer, and rudder areas (Fig. 1). Hull areas at the bow tended to have the fewest morpho-taxa although we recorded up to seven different taxa at the bow areas, inside and outside dock blocks, on three different ships. Biofouling of barges and the tug was much lower, with fewer than four taxa recorded per vessel.

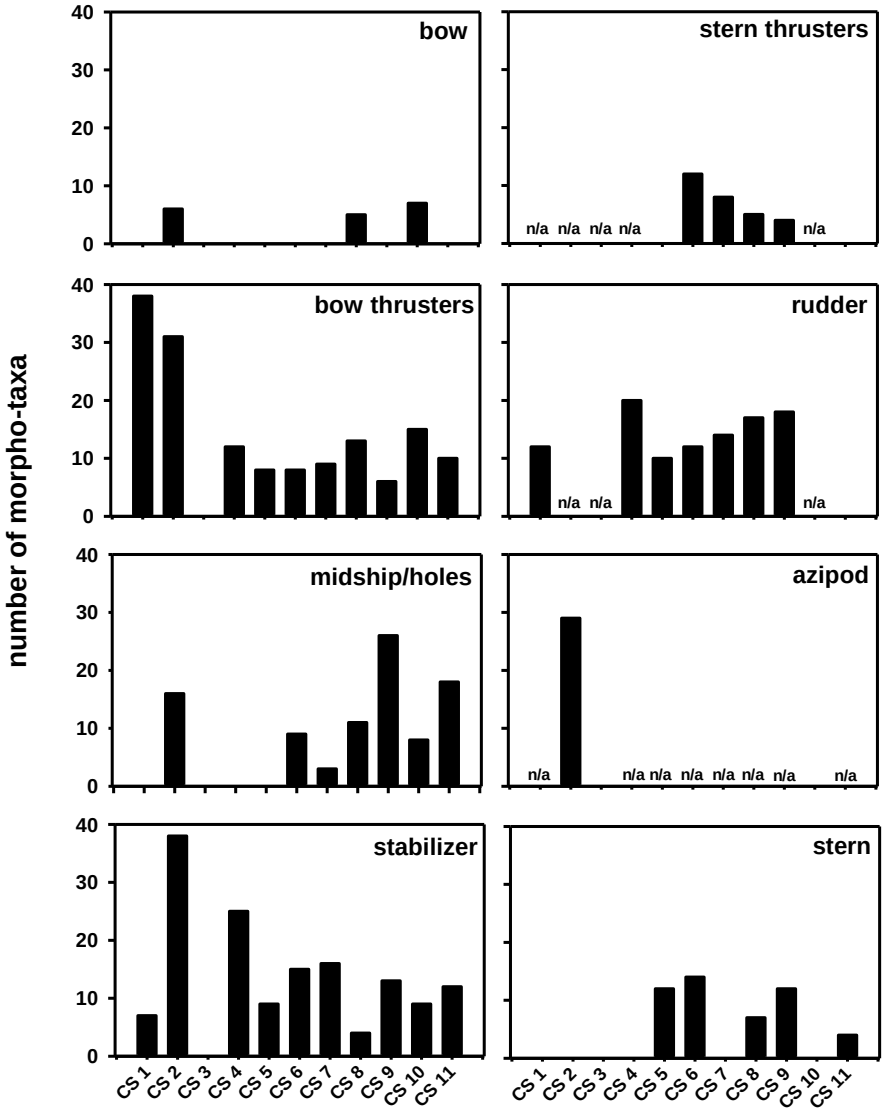

Figure 1. Biofouling morpho-taxa richness on cruise ships. The number of distinct taxa collected across eight hull locations on eleven cruise ships is plotted. For some vessels, we were unable to examine certain areas or they did not exist (e.g. there are no rudders on ships with azimuth propulsion), and these are indicated (n/a).

Analysis of organism condition is a multi-step process and includes information from initial sorting of samples and subsequent (more detailed) laboratory analyses. The categories of organisms include:

1) Live organisms that are in apparent good condition. These individuals or colonies are characterized by undamaged soft-tissue and movement of either the entire organism (mobile species) or internal parts of sessile species (e.g. filter feeding appendages). Notes are also made of animals that are live but apparently expiring (e.g. moving cilia in the gills of bivalves and tubeworms with minimal response).
2) Freshly dead organisms with soft-tissue largely present but were not moving or showing any signs of life. Mortality of these organisms may have occurred during sampling (from scraping samples or holding periods after sampling) or they may have expired during the preceding voyage. Discriminating between these can only occur after species-level identifications are completed and comparisons within- and among-ships are made for each species.
3) Dead organisms for which soft-parts were largely or entirely missing and hard parts remain. Only intact organisms were counted, fragments of organisms were not included. Our final report will evaluate the bias associated with this information because of morphological differences among taxa. Several organisms with external shells provide a live-dead comparison but soft-bodied individuals do not leave a 'dead' signal. Some hard-shell dead organisms are also more likely to leave an intact specimen compared to others, depending on their attachment type. Another concern is evaluating how representative live versus dead comparisons are of the ship as a whole (e.g. many dead organisms tend to break up upon collection making it more likely that we would under-estimate the proportion of dead individuals on a ship). It may be possible to evaluate this based on images.
4) Ovigerous organisms were also noted during initial sampling but will be evaluated in greater detail in the laboratory using preserved specimens. Focal taxa include barnacles, whose egg masses can often be seen initially and have been observed to spawn, and mobile crustaceans whose egg masses are carried by females. Initial sorting has revealed many ovigerous individuals from five of the 14 vessels examined, but we expect this number to increase upon completion of further lab work.
5) Further lab analysis will attempt to utilize published condition indices for selected taxa to provide an additional evaluation of organism viability.

Initial sorting of 19,842 barnacles from all vessels revealed that live proportions ranged from a high of 98% to a low of 33% per ship. We have also processed 4811 bivalves (primarily mussels) and 98% were live, although six vessels with few individuals (<50) had between 14% and 94% live individuals.

Parasite analyses

The protocol for analysis of parasites, focusing on bivalves (particularly mussels), has been developed and will be conducted with the aid of collaborators with extensive

experience in bivalve parasitology. Only one vessel sampled so far, *Cruise Ship 2*, has had bivalves large and numerous enough for parasite analysis. The first screening of these samples revealed a 30% infection rate with copepods. Additional analyses of these animals will determine further parasite and pathogen loads, including histological sampling for possible protist or trematode infection. In addition to further sampling of mussels on vessels, we will also incorporate some previous ship samples into this component of the project. For example, we have mussel collections from three trans-oceanic containerships that will be added to the parasite and pathogen analysis.

Evaluation of Progress & Next Steps

Satisfactory progress has been made so far in terms of numbers of vessels sampled and initial processing of samples. We have been hindered by not getting access to vessels on sampling days when pre-arranged access had already been granted. This issue has been largely resolved because participating shipping companies have been very helpful and communicative since the earlier incidents. Unforeseen diving conditions (e.g. excess current and turbidity) have also been a problem, but only on two occasions.

Another hindrance is the lack of bivalves within biofouling communities of ships. The scarcity of bivalves on otherwise well-fouled vessels provides insufficient material for mussel pathogen analysis. At least twenty individuals of > 20cm (per ship) are required for pathogen analysis and we have encountered only one vessel that fit these criteria. Mussels have been sampled on other vessels, but they were not large enough to process for parasites. We will endeavor to increase our sample size for this component of the project, by sampling additional ships and including mussels from previous sampling efforts.

Work in the coming months will focus on additional sampling of ships, most likely in LA/Long Beach. We are currently planning our next field trip, which may occur in October, to take advantage of the end-of-season transition for several cruise ships. Our aim is to reach 20 sampled vessels during this time, with funds remaining for an additional sampling effort later in 2010 or in early 2011.

Lab work will continue with identification of voucher specimens, analyses of photographs, and quantifying reproductive condition of organisms. We will provide measures of richness, extent and condition for all vessels, combining these data with recent SLC-funded work to provide comparisons among studies in the final report. Evaluations of reproductive status will focus on random sub-sampling of selected taxa from sample collections for comparison among ships and among taxa. This will provide estimates of these organisms ability to successfully 'release' from a ship and be introduced to a port area, which may perhaps be a truer estimate of propagule pressure from biofouling. Finally, we will work with colleagues from the 'mussel watch' screening program to assess parasite prevalence and transfer on ships, the first study (to our knowledge) to directly address parasites associated with biofouling vectors.

Literature cited:

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